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Strategies to develop a prophylaxis for the prevention of HPA-1a immunization and fetal and neonatal alloimmune thrombocytopenia

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ABSTRACT

Anti-HPA-1a-antibodies are the main cause of fetal and neonatal alloimmune thrombocytopenia (FNAIT) which may result in intracranial hemorrhage (ICH) and death among fetuses and newborns. Advances in understanding the pathogenesis of FNAIT and proof of concept for prophylaxis to prevent immunization suggest that development of hyperimmune anti-HPA-1a IgG aimed at preventing immunization against HPA-1a and FNAIT is feasible. Anti-HPA-1a IgG can be obtained either by isolating immunoglobulin from already-immunized women or by development of monoclonal anti-HPA-1a antibodies.

Here we discuss recent advances that may lead to the development of a prenatal and postnatal prophylactic treatment for the prevention of HPA-1a-associated FNAIT and life-threatening FNAIT-induced complications.

1. Introduction

Platelet integrins are carriers for multiallelic determinants. These antigens are commonly referred to as platelet specific alloantigens or human platelet antigens (HPAs) despite the fact that some of them are present to a lesser extent on other types of cells such as endothelial cells, lymphocytes, and placental trophoblasts. These platelet antigens play an important role in immune-mediated platelet disorders. Alloimmunization mainly occurs in relation to pregnancy or platelet transfusion. Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is suspected when a fetus or newborn is found to have thrombocytopenia, either incidentally or because of unexpected bleeding signs and symptoms. FNAIT is confirmed after identification of maternal platelet specific antibodies against fetal antigens (not expressed in the mother). In Caucasians, anti-HPA-1a antibodies are of particular interest because they account for about 85 % of all cases of severe FNAIT [1]. Severe anti-HPA-1a antibody-induced FNAIT occurs in approximately one of 1800 pregnancies [2]. Intrauterine death or neurological sequelae have been reported to occur in approximately one per 10 000 newborns [3]. In Europe, this translates into approximately 500 infants per year and in the USA approximately 400 infants per year.

Prophylactic treatment was previously not considered for FNAIT because it was generally believed that immunization against HPA-1a mainly occurred during the first incompatible pregnancy. Results from two large prospective clinical studies indicated however, that the majority of HPA-1a immunizations had occurred in connection with delivery in a previous pregnancy [4,5]. This was confirmed in a large Norwegian prospective clinical trial [2]. In the Norwegian study it was

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shown that 75 % of all cases of HPA-1a alloimmunization occurred in relation to delivery rather than during pregnancy [6]. The remaining 25 % of cases of alloimmunization occurred during an incompatible pregnancy. The realization that alloimmunization mainly occurs after delivery suggested that HPA-1a alloimmunization may be prevented in a manner similar to the prevention of RhD immunization and hemolytic disease of fetus and newborn (HDFN) [7]. For more than 5 decades, anti-D immunoglobulin has successfully been used to prevent HDFN. Before the implementation of prophylaxis against anti-D, approximately 15 % of RhD negative women became anti-D immunized, which declined to 1 % after the introduction of post-partum prophylaxis and 0.2 % when antenatal prophylaxis was added [8].

Heyu Ni and co-workers have developed a murine model, using β3 integrin (GPIIIa) deficient mice (β3-/-), that recapitulate the clinical manifestations of FNAIT [9]. The availability of an animal model of FNAIT made it possible to investigate if prophylactic treatment is feasible to prevent thrombocytopenia and bleeding symptoms caused by anti-platelet antibodies. This model was applied in a study, where female β3 integrin (GPIIIa) deficient mice were transfused with β3 positive platelets, with or without a separate injection of prophylaxis (anti-β3 antibodies) to simulate fetal-maternal bleeding (FMB). Two weeks later, the females were bred with WT BALB/c mice (β3 incompatible pregnancy). Pup platelet counts, birth weight and signs of bleeding were recorded within 24 h after delivery. This proof of concept study successfully demonstrated that prophylactic treatment with an anti-β3 antibody could prevent both maternal immunization and thrombocytopenia and bleeding complications in the pubs [10].

Although the majority of HPA-1a immunizations appear to occur during delivery, the prospective screening studies [2,4,5] reported severe FNAIT cases also in first pregnancies. Recently, a study by Jin et al. [11] reported that 60 % of affected FNAIT pregnancies were first pregnancies including 71 % of first pregnancies complicated by fetal ICH. The ICH's occurred in the second half of the second trimester and early third trimester in many of the cases. Results from this retrospective study, that included a selected group of women, still needs to be confirmed by a prospective study. However, this finding demonstrates that in order to prevent all severe FNAIT cases, both antenatal and postnatal prophylaxis is required.

In order to identify pregnant women at risk for immunization, and in need of prophylactic treatment, pregnant women need to be HPA-1a typed early in pregnancy. This could be done by screening for HPA-1a in the same sample used for RhD typing. Both phenotyping and genotyping assays can be configured for high through-put screening assays [12,13]. Further stratification, to identify high risk pregnancy may also be included in a screening program. Such a stratification should include HLA-DRB3*01:01 typing, since the risk of HPA-1a-immunisation is 25 times higher in HLA-DRB3*01:01 positive women as opposed to women lacking this HLA allele [14], and because the risk of severe neonatal outcome is very low in those few HLA-DRB3*01:01 negative women who become HPA-1a-immunized [15].

In the current review we discuss recent advances that may lead to the development of a prenatal and postnatal prophylactic treatment for the prevention of HPA-1a-associated FNAIT and life-threatening FNAIT-induced complications.

2. Collection of plasma and manufacture of hyperimmune anti-HPA-1a IgG, NAITgam

In 2012 the EU-funded PROFNAIT project was established with the aim of developing a hyperimmune anti-HPA-1a IgG prophylaxis for prevention of HPA-1a-immunization and FNAIT. The PROFNAIT consortium consisted of eleven Northern European hospitals, blood banks and companies with experience and key expertise in FNAIT and drug development (Fig. 1). In addition, strong collaboration was entered with NAITbabies (www.NAITbabies.com), a patient organization run by families affected by FNAIT, several US plasma centers as well as the manufacturing company Emergent BioSolutions, Winnipeg, Canada.

A total of 86 female donors were identified in Germany, Norway and Sweden. The majority of these donors were FNAIT mothers (n = 69) referred to the program by their doctors, the rest were identified by screening of HPA-1a negative donors at German Red Cross. Utilization of the donated EU plasma was highly desirable, but challenging since only a few FDA-compliant manufacturers allowed EU plasma into their facilities, including, Emergent BioSolutions. For this reason, plasma collection was initiated in the US. NAITbabies encouraged their HPA-1a-immunized members to sign up as plasma donors on PROFNAIT's webpage, as did centers involved in the care of affected women. In the US more than 100 alloimmunized women volunteered to donate plasma. An informed consent was obtained from all recruited donors. In addition, a high number of female plasma donors were screened for anti-HPA-1a antibodies and enrolled in the NAITgam donation program.

For a donor to be included in the NATIgam screening program, the donor had to be HPA-1bb and have anti-HPA-1a antibody level > 10 IU/mL. Donors with anti-Human Neutrophil Antibodies (HNA), platelet activating HPA-1a antibodies or alloantibodies towards red blood cells (RBC) were excluded. One European donor had anti-HNA antibodies and platelet activating HPA-1a antibodies and was excluded from the donation program. None of the female donors had alloantibodies against RBC. The anti-HPA 1a antibody level in the individual donors fell to approximately 50 % of the starting level due to frequent donations. Mean anti-HPA-1a antibody level in the whole pool was 40 IU/mL (SEM = 7.8 IU/mL). Thus, the final plasma pool used for manufacture of NAITgam had no alloantibodies against RBC, no anti-HNA or platelet activating HPA-1a antibodies. However, low levels of anti-HLA I (titer 1) were detected.

The glycosylation profile of the IgG antibody's Fc fragment, particularly the absence of core fucosylation, which is normally present in all IgG, is known to be essential for Fc receptor-mediated activity [16–18]. Kapur et al. have recently demonstrated that HPA-1a IgG antibodies in immunized women have very low levels of fucose as compared with total IgG. Similarly, in RhD-immunized women, the IgG antibodies against the RhD antigen have also low levels of fucose, which is also reflected in the polyclonal prophylactic anti-D drug products [19–21]. The level of anti-HPA-1a IgG1 fucosylation in FNAIT pregnancies has been shown to be associated with the platelet counts in the new-born [21]. A follow-up study showed that this alteration in fucosylation of anti-HPA-1a seems to occur early in the immune response (first/second pregnancy) and then be extremely stable over time [22].

The glycosylation profiles of the donors from HPA-1a-immunized women were investigated (both EU and US donors), and we found no differences in fucosylation patterns between anti-HPA-1a IgG1 isolated from donors identified by screening (unknown FNAIT history) compared to anti-HPA-1a isolated from donors who have given birth to children with clinical bleeding symptoms (FNAIT mothers). Both groups had a decreased fucosylation in the total IgG (84 %) compared to the healthy population (94 %) (Table 1).

All plasma was collected and tested in compliance with FDA and EMA guidelines aimed for production of NAITgam both for the European and US markets. In June 2019, Rallybio, a US biotech company dedicated to identify and accelerate development of life-transforming therapies for patients with severe and rare disorders, acquired the orphan drug program for NAITgam from Prophylix AS, and will proceed with safety and dose finding clinical phase I/II trials in 2020.

NAITgam was manufactured by the same process as used for by Emergent BioSolution for production of their intravenous immunoglobulin (IVIg) products. Thus, NAITgam will be similar to other hyperimmune products manufactured by Emergent BioSolution, except for the antibody specificity. This means that one would expect that these hyperimmune drug products would be representative for the safety profile of NAITgam. Broad experience in humans with

Fig. 1. The PROFNAIT Consortium.

intravenous administration of IgG drug products has demonstrated a favorable safety profile of this class of products As an example, WinRho® (IV anti-D) has been on the market for more than 30 years first for the prevention of HDFN and later for treatment of immune thrombocytopenia (ITP) [23].

The World Health Organization (WHO) has determined that 125 IU anti-D IgG is the dose needed to prevent sensitization derived from one mL of fetal whole blood during pregnancy [24]. A standard dose of

Table 1
Source and pool plasma characteristics.

Hyperimmune plasma for NAITgam production	
Total Volume, (L)	84 (EU), 515 (US)
Individual anti-HPA-1a antibody level, range (IU/ml)	2-143
Percentage of fucosylated total IgG (n = 81), mean % (SEM)	84 (9)
Percentage of fucosylated anti-HPA-1a IgG (n = 81), mean % (SEM)	37 (4)

1500 IU would generally suppress an immunization after FMB of up to 30 mL of fetal whole blood [25,26]. It is worthwhile mentioning that even though a 30 mL FMB is used as a guidance for optimal prevention of maternal immunization, it has been estimated that FMB, at the time of delivery, is limited to a maximum of 1 mL fetal red cells in 98 % of women [27]. Further, from anti-D studies we know that it is not necessary to obtain full saturation of the antigenic sites to obtain antibody-mediated immune suppression (AMIS) [28], which most likely could also be the case for anti-HPA-1a. *In vitro* studies have shown that approximately 0.5 IU/ml anti-HPA 1a antibodies saturate approximately 10 % of HPA 1a on HPA 1ab platelets. In an *in vivo* situation this would be equal to a dose of 2000 IU. The optimal dose of NAITgam, in order to induce rapid clearance, will be determined in a clinical phase II trial in 2020.

3. Possible mechanism of action

Immunization against HPA-1a occurs when a woman is exposed to fetal platelet antigens, most probably due to a FMB during gestation or at delivery. The risk of FMB is low during the first two trimesters but increases in cases of early spontaneous or provoked abortion. During last trimester, FMB has been shown to occur in approximately 6–7 % of all pregnancies [29]. Fetal blood is commonly found in maternal circulation and detected in around 96 % of normal deliveries after delivery [30]. It has also been speculated if microvesicles due to shedding of trophoblasts could be an alternative source for immunization against HPA-1a [31] or that immunization could be caused by invasive trophoblast cells that express integrin β3, which harbor the HPA-1a antigen. Maternal anti-HPA-1a antibodies directed against fetal HPA-1a cross the placenta and bind fetal platelets, which in turn are removed by splenic macrophages through antibody mediated phagocytosis [32] leaving the fetus and/or newborn thrombocytopenic and at risk for bleeding. Additionally, maternal anti-HPA-1a antibodies binding to megakaryocytes are believed to increase the severity of the thrombocytopenia [33] and binding to developing endothelial cells have also been suggested to result in bleeding in the fetus and newborn [34].

The mechanisms of hyperimmune anti-HPA-1a to prevent immunization against HPA-1a would likely be analogous to the proposed mechanisms of hyperimmune anti-D. Explanations for AMIS have been discussed for many years [35], but AMIS is still not fully understood. One possible mechanism is that that anti-D-sensitized fetal RBC will be subject to phagocytosis by the maternal mononuclear phagocytic system before the mother starts an antibody response. Accordingly, it is believed that anti-HPA-1a antibodies, when administered to HPA-1a negative women, will sensitize fetal platelets and remove fetal HPA-1a positive platelets, thereby preventing maternal HPA-1a-immunization and FNAIT. Several studies supporting this hypothesis have shown that administration of antibodies against RBC or platelets lead to rapid clearance of the targeted cells [36–39]. Ghevaert et al. showed that binding of a monoclonal antibody specific to HPA-1a cleared HPA-1ab positive platelets within 2 h. However, alternative mechanisms, such as (partial) epitope masking, antigen-modulation and/or co-engagement of the B cell receptor with the inhibitory Fc-receptor FcγRIIb can't be excluded [40].

The HPA-1a antigen is located on the β3 integrin. Platelets express both αIIbβ3 (fibrinogen receptor) and αvβ3 (vitronectin receptor). In addition, αvβ3 is present on both endothelial cells [41,42] and trophoblasts [43]. Studies on maternal antibody response against HPA-1a have shown that the immunoglobulins are heterogeneous and targeted binding sites differ from one individual to another [34,44–46]. It has been shown that some anti-HPA-1a antibodies bind to an epitope comprised solely of the amino-terminus on β3, while others bind to more complex determinants involving both the β3 and αIIb integrins [45,46]. In addition, Santoso and co-workers [34] have identified a third sub-type of HPA-1a antibodies that react with the αvβ3 integrin complex. This sub-type of anti-HPA-1a was mainly present in sera from women who had given birth to children with ICH, indicating that the anti-HPA-1a binding pattern is important for clinical outcome. The fact that there are individual differences in the anti-HPA-1a antibody pattern might also be an important aspect in effect of the prophylaxis, and support the idea that hyperimmune anti-HPA-1a prophylaxis generated from several donors, to ensure a broad binding pattern towards HPA-1a, could be important.

An alternative to plasma derived hyperimmune anti-HPA-1a prophylaxis could be the use of a monoclonal anti-HPA-1a antibody. The advantages of monoclonal antibodies are their homogeneity and consistency. However, the monospecificity of such antibodies could limit their usefulness, as binding to a single HPA-1a epitope may not sufficiently deliver the prophylactic preventive function. Polyclonal antibodies, on the other hand, are heterogeneous and recognize several antigenic epitopes. The heterogeneous nature of polyclonal antibodies, however, also makes them more prone to batch-to-batch variability, but this can largely be overcome using sizable pools of donors, which has a proven to be a successful strategy for RhD-prophylactic products. Another key advantage of using a monoclonal antibody is that once the desired production systems has been established, anti-HPA-1a IgG can be generated as a constant and renewable resource. Further, the concentration and purity levels of specific antibodies can be made higher in drug products based on monoclonal antibodies; typically monoclonal antibodies generated in specialized cell cultures are frequently 10-fold higher in concentration than polyclonal antibodies, and of much higher purity [47]. Many of the disadvantages of monoclonal antibodies may possible be overcome by pooling multiple clones that recognize different HPA-1a epitopes. However, it is difficult, time consuming and expensive to identify multiple monoclonal antibodies of a separate, desired specificity.

A fully human monoclonal anti-HPA-1a antibody has been generated from an immunized woman who had a child with FNAIT. This antibody, which binds both to the αvβ3 integrin as well as the αIIbβ3 integrin [48], the two different HPA-1a epitopes known to be important for severe FNAIT [34,46], is a good candidate for future prophylaxis of HPA-1a-immunization and FNAIT.

4. Conclusion

Currently there is a compelling need to develop new effective therapeutic treatments to prevent FNAIT and FNAIT induced complications. Collecting plasma and manufacturing of the first GMP batch of hyperimmune anti-HPA-1a IgG is a great step forward in the fight against FNAIT. Prophylactic treatment has great potential and may be a clinical reality in the near future. Prenatal prophylactic treatment would be superior to a single postpartum treatment, since immunization and complications due to severe FNAIT would be prevented also in first pregnancies.

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Declaration of Competing Interest

MK and BS belong to a group of founders and owners of Prophylix AS, a Norwegian biotech company that produced hyperimmune anti-HPA-1a IgG (NAITgam) April 2019. Further, MK and JB are consultants for Rallybio, a US biotech company that will continue development of NAITgam for prevention FNAIT.

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